(11-7-98)

MRID No. 444577-31

# DATA EVALUATION RECORD
## § 72-2 - ACUTE $EC_{50}$ TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Prohexadione Calcium — PC Code No.: 112600

2. **TEST MATERIAL:** BX-112 — Purity: 93.3%

3. **CITATION:**

| | |
|---|---|
| Authors: | M.T. Douglas, R.W.S. Hall, and I.A. Macdonald |
| Title: | The Acute Toxicity of BX-112 to *Daphnia magna* |
| Study Completion Date: | February 3, 1997 |
| Laboratory: | Huntingdon Research Centre Ltd., Cambridgeshire, England |
| Sponsor: | BASF Corporation, Research Triangle Park, NC |
| Laboratory Report ID: | KCI 37(c)/90531 |
| MRID No.: | 444577-31 |
| DP Barcode: | D245631 |

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

**Signature:** Karl Bullock **Date:** 7/7/98

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

**Signature:** P. Kosalwat **Date:** 7/7/98

5. **APPROVED BY:**

**Signature:** **Date:** 11/17/98

6. **STUDY PARAMETERS:**

| | |
|---|---|
| **Age of Test Organism:** | ≤24 hours |
| **Definitive Test Duration:** | 48 hours |
| **Study Method:** | Static |
| **Type of Concentrations:** | Mean measured |

7. **CONCLUSIONS:** This study is scientifically sound ~~and~~ but does not fulfill ~~the~~ guideline requirements for a core level study. The 48-hour $EC_{50}$ value of >100 ppm nominal (>90.0 ppm ai mean measured concentration) classifies BX-112 as practically non-toxic to *Daphnia magna*. The NOEC was determined to be 90.0 ppm ai.

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Bym 12/2000

**Results Synopsis**

$EC_{50}$: >90.0 ppm ai 95% C.I.: N/A

NOEC: 90.0 ppm ai Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

**A. Classification:** ~~Core~~ Supplemental* BJM 6/14/2000

**B. Rationale:** Fulfills the guideline requirements.

**C. Repairability:** N/A.

9. **GUIDELINE DEVIATIONS:**

1. The reported hardness (350 mg/L as $CaCO_3$) and pH (7.8-8.4) were higher than recommended (hardness: 40 - 200 mg/L as $CaCO_3$; pH: 7.2-7.6).

2. Dilution water was dechlorinated tap water.

3. Temperature was measured at test initiation and daily thereafter; guideline protocol recommends continuous temperature monitoring.

4. The test concentration was slightly below the required 100 ppm ai.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

**A. Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species** Preferred species is *Daphnia magna* | *Daphnia magna* |
| **All organisms are approximately the same size and weight?** | Not reported |

| Guideline Criteria | Reported Information |
|---|---|
| **Life Stage**<br>Daphnids: 1st instar (<24 h).<br>Amphipods, stoneflies, and mayflies: 2nd instar.<br>Midges: 2nd & 3rd instar. | 1st instar (≤24 h) |
| **Supplier** | In-house culture originating from IRCHA, France |
| **All organisms from the same source?** | Yes |

B. Source/Acclimation

| Guideline Criteria | Reported Information |
|---|---|
| **Acclimation Period**<br>Minimum 7 days | Cultures were maintained under conditions similar to testing. |
| **Wild caught organisms were quarantined for 7 days?** | N/A |
| **Were there signs of disease or injury?** | Not reported |
| **If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?** | N/A |
| **Feeding**<br>No feeding during the study. | No feeding during the study |
| **Pretest Mortality**<br>No more than 3% mortality 48 hours prior to testing. | Not reported |

C. Test System

| Guideline Criteria | Reported Information |
|---|---|
| **Source of dilution water**<br>Soft reconstituted water or water from a natural source, **not** dechlorinated tap water. | Dechlorinated and aged tap water |

| Guideline Criteria | Reported Information |
|---|---|
| **Does water support test animals without observable signs of stress?** | Yes |
| **Water Temperature**<br>Daphnia: 20°C<br>Amphipods and mayflies: 17°C<br>Midges and mayflies: 22°C<br>Stoneflies: 12°C | 21.0°C |
| **pH**<br>Prefer 7.2 to 7.6. | 7.8 - 8.4 |
| **Dissolved Oxygen**<br>Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%. | ≥83% during the test |
| **Total Hardness**<br>Prefer 40 to 200 mg/L as $CaCO_3$. | 350 mg/L as $CaCO_3$ |
| **Test Aquaria**<br>1. Material:<br>Glass or stainless steel.<br>2. Size:<br>250 mL (daphnids and midges) or 3.9 L (1 gal).<br>3. Fill volume:<br>200 mL (daphnids and midges) or 2-3 L. | <br>Glass<br><br>Not reported<br><br>200 mL |
| **Type of Dilution System**<br>Must provide reproducible supply of toxicant. | N/A |
| **Flow Rate**<br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period. | N/A |
| **Biomass Loading Rate**<br>Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day. | One daphnid per 20 mL of solution. |
| **Photoperiod**<br>16 hours light, 8 hours dark. | 16 hours light, 8 hours dark |

| Guideline Criteria | Reported Information |
|---|---|
| **Solvents**<br>Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. | None |

D. Test Design

| Guideline Criteria | Reported Information |
|---|---|
| **Range Finding Test**<br>If $EC_{50}$ >100 mg/L, then no definitive test is required. | No range-finding test was conducted. |
| **Nominal Concentrations of Definitive Test**<br>Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one. | Negative control and 100 mg/L (93.3 mg ai/L). |
| **Number of Test Organisms**<br>Minimum 20/level, may be divided among containers. | 40 per level, 10 per replicate |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Water Parameter Measurements**<br>1. Temperature<br>Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C.<br>2. DO and pH<br>Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control. | Temperature was measured at test initiation, at 24 hours, and at test termination.<br><br>DO and pH were measured at test initiation and termination. |

| Guideline Criteria | Reported Information |
|---|---|
| **Chemical Analysis** Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used | Solutions were collected at 0 and 48 hours and analyzed using HPLC. |

**12. REPORTED RESULTS:**

**A. General Results**

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes |
| **Control Mortality** Static: ≤10% Flow-through: ≤5% | 0% mortality in the control |
| **Percent Recovery of Chemical** | Range 92 - 100% |
| **Raw data included?** | Yes |

Mortality/Immobilization

| Nominal Concentration (mg ai/L) | Mean measured Concentration (mg ai/L) | Number of Daphnids | Cumulative Number Immobile/Dead | |
|---|---|---|---|---|
| | | | 24-hr | 48-hr |
| Control | <0.25 | 40 | 0 | 0 |
| 93.3 | 90.0 | 40 | 0 | 0 |

Other Significant Results: No sublethal signs of toxicity were reported.

**B. Statistical Results:**

Method: Visual observation

48-hr $EC_{50}$: >100 mg/L 95% C.I.: N/A

Probit Slope: N/A NOEC: 100 mg/L

13. **VERIFICATION OF STATISTICAL RESULTS:**

Method: Visual observation

48-hr $EC_{50}$: >90.0 ppm ai 95% C.I.: N/A

Probit Slope: N/A NOEC: 90.0 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the guideline requirements, and can be classified as Supplemental. Although the test material was not tested up to 100 ppm ai, the reviewer does not believe that increasing the concentration by 10 ppm ai would have changed the outcome of this study. The 48-hour $EC_{50}$ for *Daphnia magna* exposed to BX-112 was >100 ppm nominal or >90.0 ppm ai mean measured concentration, which classifies BX-112 as practically non-toxic to the daphnid. The NOEC was determined to be 90.0 ppm ai.